Reference: *Biol. Bull.* **183**: 463–475. (December, 1992)

Nervous System of the Tornaria Larva (Hemichordata: Enteropneusta). A Histochemical and Ultrastructural Study

S. SH. DAUTOV[1] AND L. P. NEZLIN[2]

[1]*Institute of Marine Biology FEBS Academy of Sciences of Russia 17, Palchevsky str., 690041 Vladivostok, Russia, and* [2]*Institute of Developmental Biology Academy of Sciences of Russia 26, Vavilov str., 117808 Moscow, Russia*

Abstract. Transmission electron microscopy (TEM) and histochemical approaches were used to investigate the topology and ultrastructure of the nervous system of the tornaria larva of an enteropneust, *Balanoglossus proterogonius*. Cholinesterase activity was detected in the epithelium of the pre- and postoral ciliary bands. Groups of catecholamine-containing cells (CA) were detected at the anterior tip of larva, in the ventral epidermis behind the mouth, and in the stomach wall near its junction with the intestine. Single CA neurons were detected in the telotroch epithelium. Axon tracts are described in ciliary band epithelia. At the base of the aboral plate, epithelial nerve cells form a ganglion-like cluster. Single neuron-like cells and single axons and axonal tracts were found in the epithelium of digestive tract. The data were compared with ones from the literature and with those obtained from other marine invertebrate larvae. The properties of the neural elements and their possible functions are discussed.

Introduction

The tornaria is the principle free-swimming larva of enteropneusts (Phylum Hemichordata, Class Enteropneusta). Despite some differences, all tornaria have much in common. They have three ciliary bands serving as locomotory or feeding organs: the preoral and postoral bands are used for both swimming and collecting of food particles; but the adoral band (telotroch) is used for locomotion only (Strathmann and Bonar, 1971, 1976; Strathmann *et al.*, 1972). The ventrally-located mouth leads to the digestive tract, which is divided into three parts. An apical plate with a tuft of sensory cilia and paired eyespots are located at the anterior tip of the body.

Tornaria look very much like the hypothetical ancestral echinoderm larva, the dipleurula, and they were originally described as asteroid larvae (Müller, 1850; Krohn, 1854). Later Metschnikoff (1870) showed tornariae to be the free-swimming larvae of enteropneusts. At present, tornariae and echinoderm larvae are classified together as the "dipleurula type" of larvae (Ivanova-Kazas, 1978). The neuromorphology of several echinoderm larvae has been studied with light and electron microscopy and biogenic amines have been visualized histochemically (Burke, 1978, 1983a, b; Nezlin *et al.*, 1984; Bisgrove and Burke, 1986, 1987; Burke *et al.*, 1986; Chia *et al.*, 1986; Nakajima, 1987).

The nervous system of the tornaria remains essentially unknown. Ivanova-Kazas (1978) referred to a nerve plexus under the base of the apical plate, but only the structure of the eyespots has been described in detail (Branderburger *et al.*, 1973). We present ultrastructural and histochemical evidence for the larval nervous system of tornaria and compare it to that known for larval echinoderms.

Materials and Methods

Tornaria identified as *Tornaria ancoratae* (Damas and Stiastny, 1961) were collected from plankton of Vostok Bay, Sea of Japan in September-October from a depth of 5–20 m (the majority of tornariae were collected near the bottom).

For transmission electron microscopy (TEM), larvae were fixed in a solution of 2.5% glutaraldehyde (Sigma) in 0.05 *M* cacodylate buffer (pH 7.0) with 0.142 *M* NaCl and 0.283 *M* sucrose for 1 h at 5°C, rinsed in 0.1 *M* cacodylate buffer, and postfixed in 2% osmium tetroxide

Received 9 August 1991; accepted 26 August 1992.

in 0.05 *M* cacodylate buffer with 0.205 *M* NaCl and 0.389 *M* sucrose. After dehydration in a graded series of ethanol and acetone solutions, samples were embedded in Epon-Araldite. Sections were cut with an LKB-3 Ultracut, stained with uranyl acetate followed by lead citrate, and observed with a JEOL-100B electron microscope.

Cholinesterase activity was localized by the direct thiocholine method (Koelle and Friedenwald, 1949), with acetylthiocholine iodide (Sigma) as a substrate. Catecholamine-containing cells were visualized by the glyoxilic acid-induced fluorescence technique (de la Torre and Surgeon, 1976; Sharp and Atkinson, 1980). Larvae were incubated in a solution containing 2% glyoxilic acid (Fluka) and 4% sucrose in 0.1 *M* phosphate buffer (pH 7.4, 18°C) for 10–20 min. The specimens were then placed on a glass slide, dried with a stream of hot air (80°C, 5 min), and mounted in liquid paraffin under a glass coverslip. The larvae were examined with a UV epifluorescence microscope (ML-2). To be confident about the specificity of the reaction, ascorbic acid was substituted for the glyoxilic acid in some samples.

Results

This tornaria larva has a barrel shaped body with a sharply pointed anterior end bearing paired eyespots (Fig. 1). Its triangular oral field is surrounded by the preoral ciliary band. The dorsal field is greater in area than the oral field. Tentacles are absent.

The tornariae are present in plankton from late August until late October. All of the specimens are morphologically identical, so these larvae probably belong to the same species, *Balanoglossus proterogonius* Belichov (Van-der Horst, 1933) which was described from this region of Sea of Japan. Adults of this enteropneust species have not been collected recently from Vostok Bay.

Transmission electron microscopy

A cross section of the ciliary band is shown in Figure 2. It consists of fusiform cells organized as a pseudostratified epithelium. Each cell bears on its apical surface a single cilium surrounded by a collar of microvilli. The cilium has a typical axonemal complex (9 + 2). The ciliated band is 6-12 cells wide. A nerve tract containing 15-20 axons lies between the epithelial cells and their subjacent basal lamina. Additionally, some axons occur at the base of the band outside the tract but still superjacent to the basal lamina. The axons are 0.2–1.5 μm in diameter; the cytoplasm contains microtubules, mitochondria, rough endoplasmic reticulum, and vesicles of different types. Both clear vesicles, 50 nm in diameter, and vesicles with electron-dense cores, 200 nm in diameter, are found within these axons (Figs. 3, 4). Some ciliated cells of the band have a process that enters the axonal tract. Adjacent to the tract several subepithelial neuron-like cells have

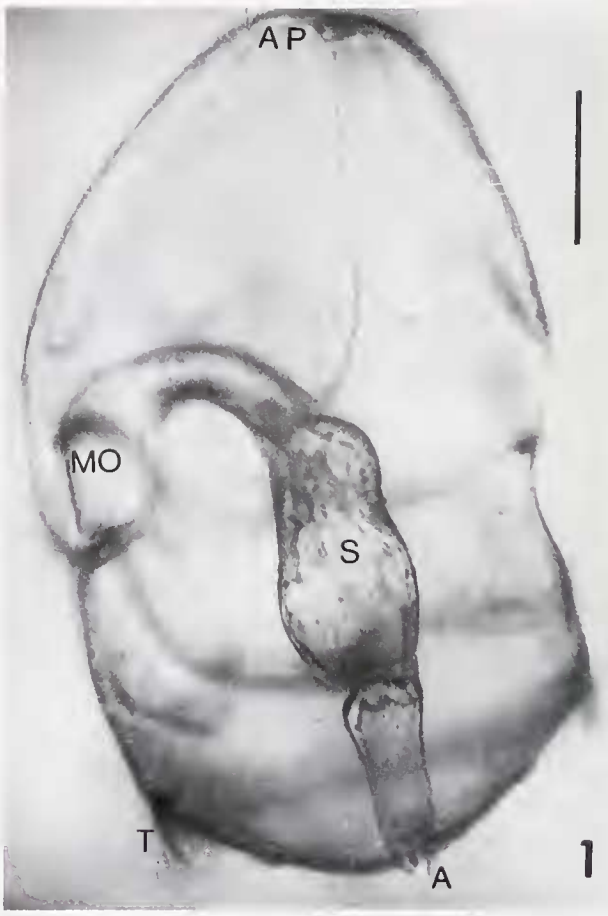


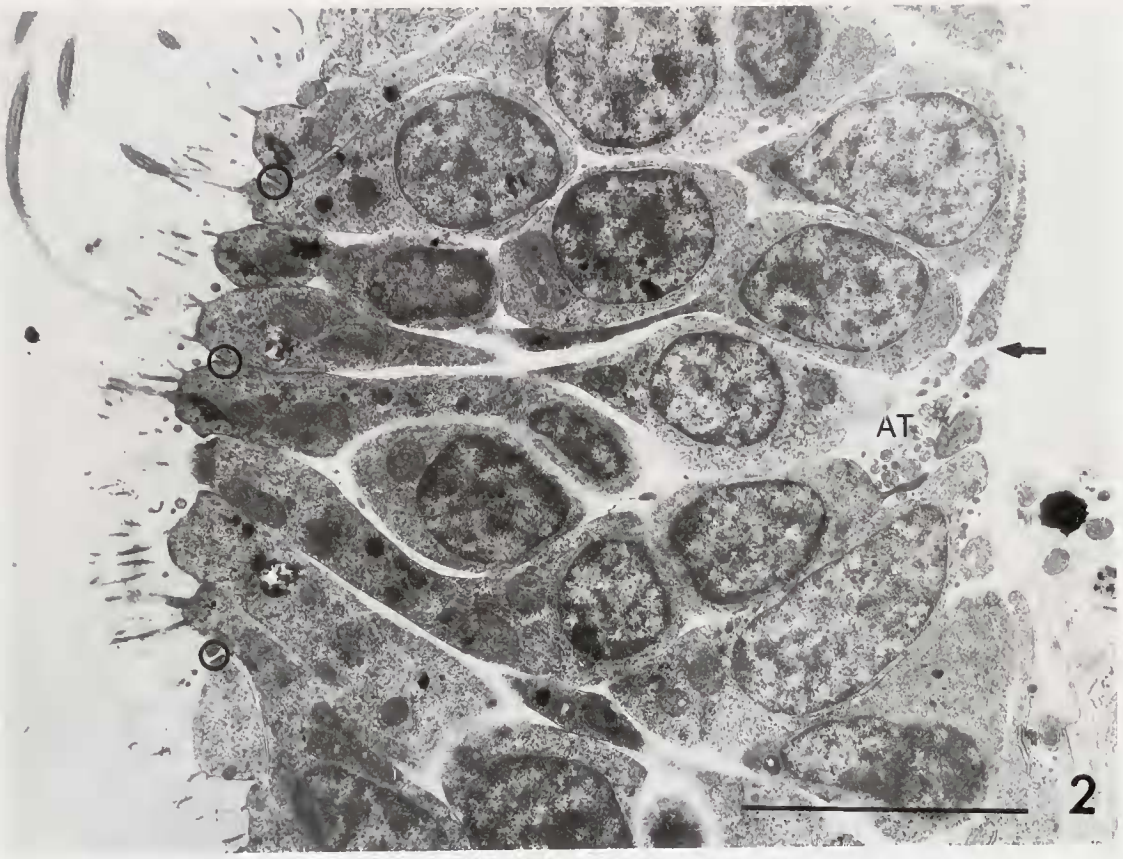
Figure 1. Left lateral view of a fully developed tornaria *Tornaria ancoratae* from plankton of Vostok Bay of Sea of Japan. A, anus; AP, apical plate; MO, mouth; S, stomach; T, telotroch. Scale bar = 100 μm.

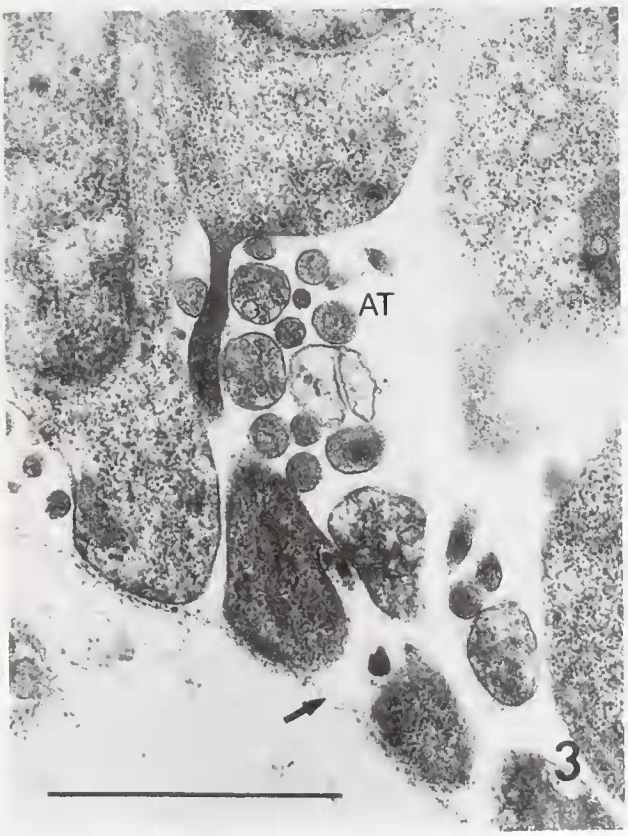
been found. These cells have no detectable cilia. They too have processes that may enter the nerve tract (Fig. 3). The apical ends of the epithelial cells are connected by tight junctions.

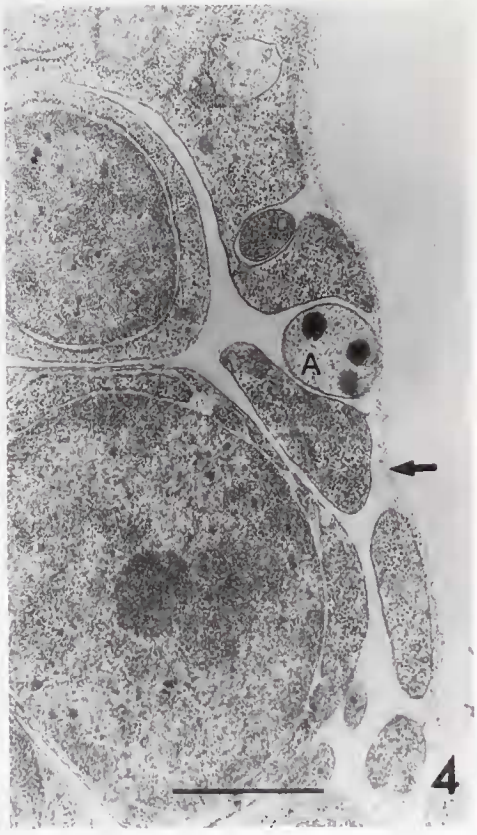
Except at the ciliary bands, the body wall is composed of a squamous epithelium. Adjacent to its base, axons are present singly or in small bundles (Figs. 5, 6). Axons can also pass within grooves, either between adjacent epithelial cells, or embedded in one of them (Figs. 7, 8).

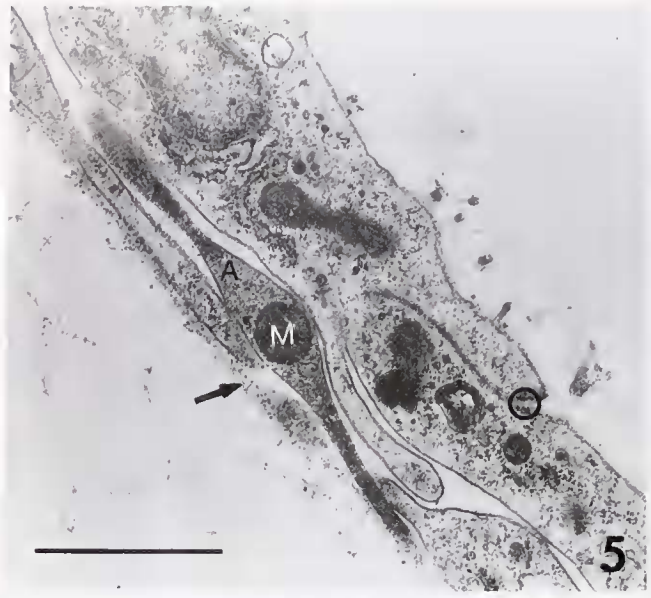
At the anterior end of the body, a thick cuboidal epithelium constitutes the aboral plate with its tuft of cilia. Here at the base of the epithelium, a cluster of nerve cells forms what appears to be a ganglionic mass (Fig. 9). The cluster is surrounded by the neurites of the nerve plexus (Fig. 10); these neurites contain mitochondria, microtubules and diverse vesicles.

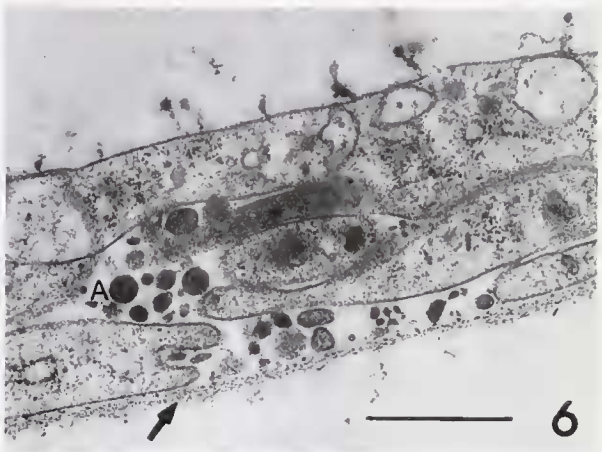
Lateral to the ganglion the epithelium becomes thinner. Here a group of special cells occurs in the epithelium (with 5–10 cilia each); they are thus quite different from the other epithelial cells (Fig. 11). They have a lobate nucleus and a rough outer surface covered with


AT
2


AT
3


A
4





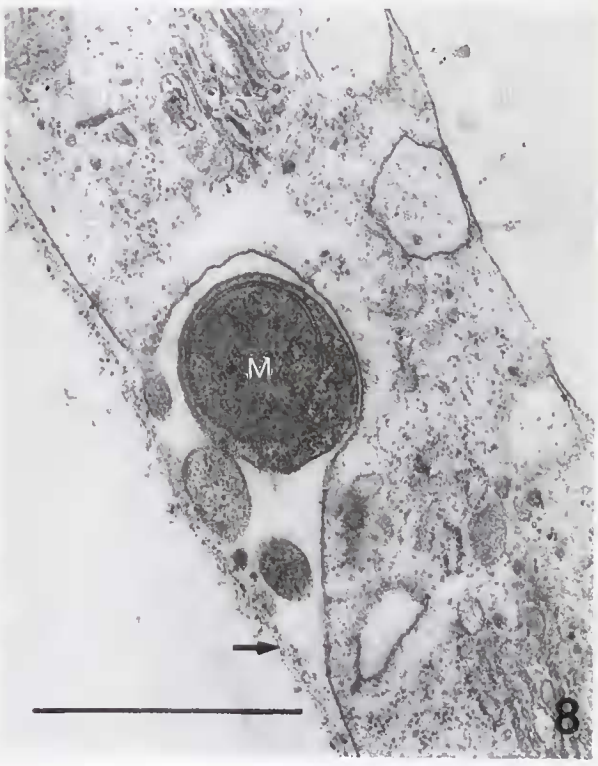


Figure 5. TEM. Longitudinal section of single axon (A) containing mitochondria (M) in the body wall outside ciliary band near basal lamina (arrow). Circle indicates tight junction. Scale bar = 1 μm.

Figure 6. TEM. Cross section of the axonal tract (A) inside the body wall epithelium. Arrow indicates a basal lamina. Scale bar = 2 μm.

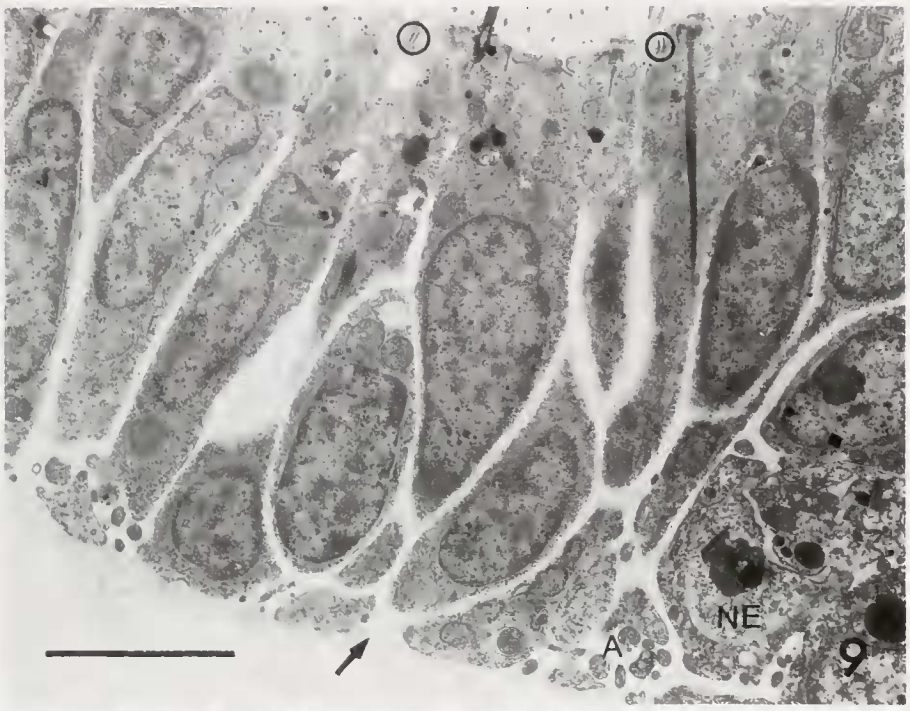
Figure 7. TEM. Two axons (large arrow) between adjacent cells of the body wall. Small arrow indicates a basal lamina. Circle indicates tight junction. Scale bar = 2 μm.

Figure 8. TEM. Several axons inside groove in inner side of the body wall cell. Larger axon contains mitochondrion (M). Arrow indicates basal lamina. Scale bar = 2 μm.

Figure 2. Transmission electron microscopy (TEM). Cross section of ciliary band with axonal tract (AT) in its base near a basal lamina (arrow). Circles indicate tight junctions. Scale bar = 5 μm.

Figure 3. TEM. Cross section of nerve (AT) in the base of ciliary band near a basal lamina (arrow). Some axons contain transparent vesicles. Basal part of epithelial cell gives process to the tract. Scale bar = 1 μm.

Figure 4. TEM. Solitary axons at the base of the ciliary band near the basal lamina (arrow). One axon (A) contains electron dense-core vesicles. Scale bar = 1 μm.



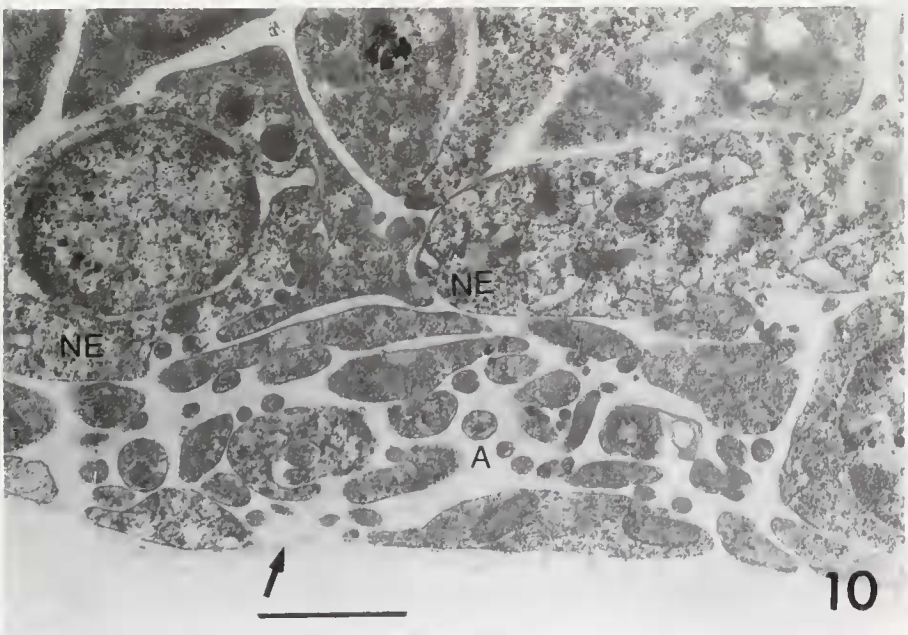


Figure 9. TEM. A part of the apical plate with a cluster of neuron-like cells (NE) and axons (A) at the base of the epithelium near a basal lamina (arrow). Circles indicate tight junctions. Scale bar = 2 μm.

Figure 10. TEM. Axons and neurons (NE) in the apical plate. Nerve plexus (A) is at the base of epithelium adjacent to basal lamina (arrow). Scale bar = 1 μm.

microvilli. In the apical region, the cytoplasm includes the Golgi complex, mitochondria and cisternae of rough endoplasmic reticulum (RER). Some cells have many large clear vacuoles. Tracts consisting of 3–5 axons each run between basal parts of these cells and the basal lamina. Around the ganglion, several axonal tracts each containing about fifty fibers, pass under the body wall (Fig. 12).

The epithelium of the esophagus is built from cuboidal multiciliated cells. Close to their bases numerous axonal tracts, of 10–20 fibers each, pass in different directions between the epithelial cells and the basal lamina (Fig. 13).

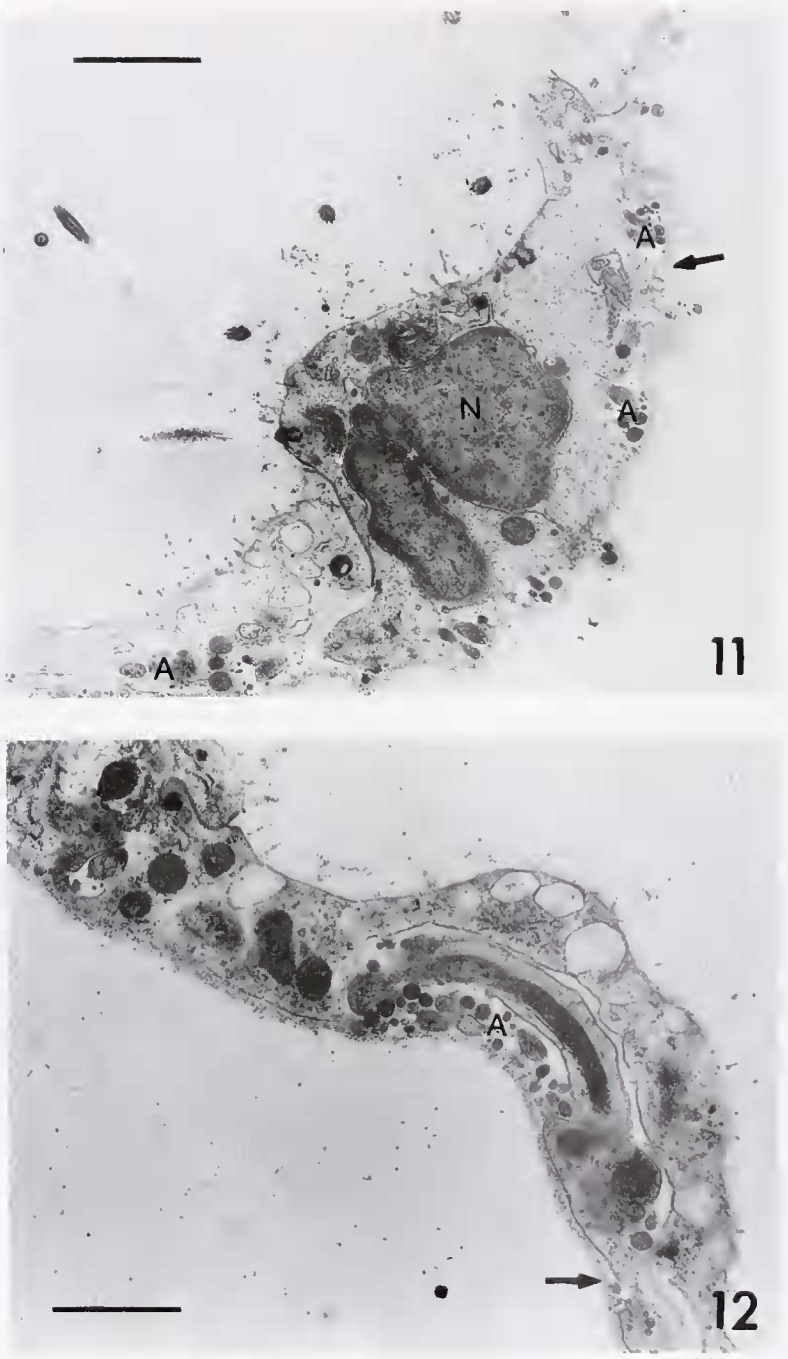


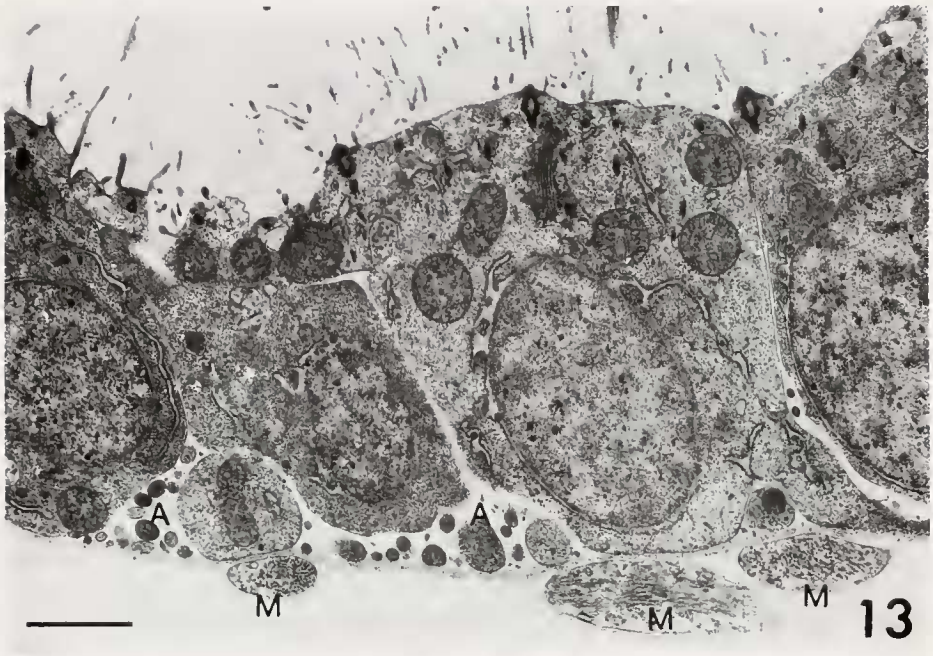
Figure 11. TEM. Epithelial cell lying laterally to the apical plate (frontal section) with lobate nucleus (N) and axonal tracts (A) at the base. Arrow indicates a basal lamina. Scale bar = 2 μm.

Figure 12. TEM. Axons (A) passing at the base of the body wall from the apical ganglion. Arrow indicates a basal lamina. Scale bar = 1 μm.

Several cells of the epithelium send processes to these traets. Behind the mouth opening a group of subepithelial cells and numerous nerve fibers are present (Fig. 14). Muscle cells, which presumably assist in swallowing of food, lie at the base of esophageal epithelium coated by the layers of a basal laminae (Figs. 13, 14).

The epithelium of the stomach consists of cells of different types. At their bases only a few solitary axons were detected (Fig. 15).

Near the junction of the stomach with the intestine are 3–4 nerve tracts (Fig. 16). Each tract contains not more than ten axons. We detected some cells where processes



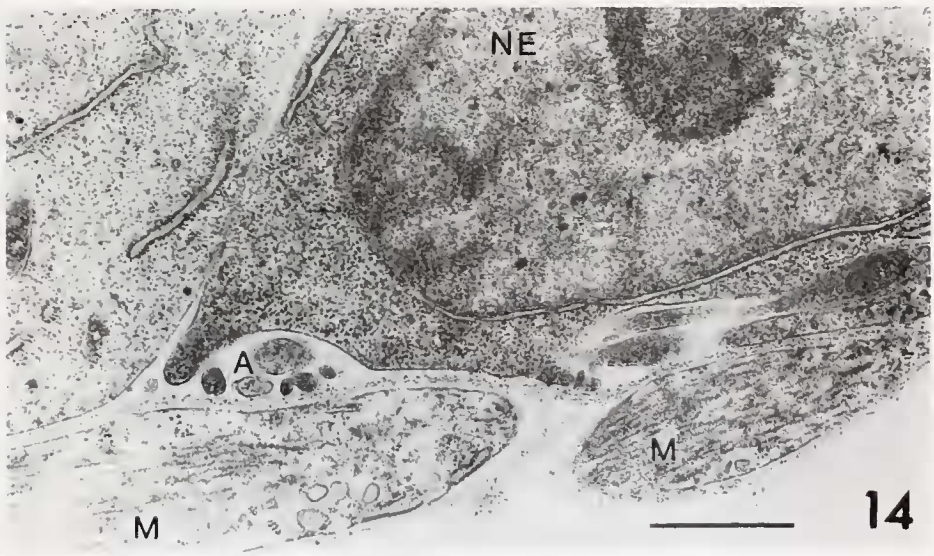


Figure 13. TEM. Longitudinal section of the esophagus showing ciliated epithelial cells, nerves (A) near the basal lamina and muscle cells (M) coated by layers of a basal lamina. Scale bar = 1 μm.

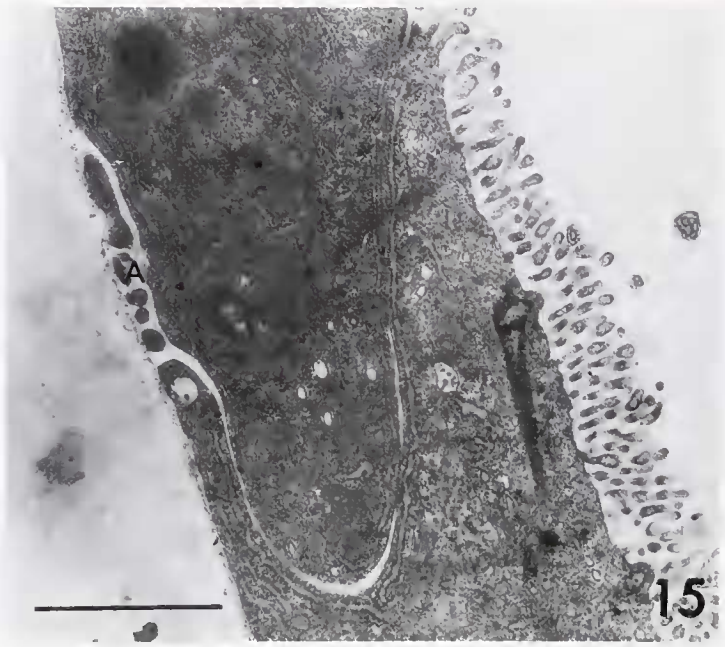
Figure 14. TEM. Neuron-like cell (NE) in the esophagus epithelium with axons (A) at its base. M, muscle cells surrounded by layers of the basal lamina. Scale bar = 2 μm.

that entered to the tract arose from the basal surface. The cytoplasm of the cells has high electron density and includes numerous mitochondria, RER, and vesicles.

The telotroch consists of multiciliated epithelial cells (Fig. 17). In addition to cilia, these cells have microvilli on their external surfaces. At the base of the telotroch, several tracts consisting of a few axons each run the length. Some cells with a very electron-dense cytoplasm lie in the epithelium. Processes extend from the base of these cells to the nerve tract. Another ciliary ring is situated between the telotroch and the anal opening. It consists of flat multiciliated cells with very few microvilli on their outer surfaces. Few axons run along this ring in grooves between adjacent cells (Fig. 18).

Histochemistry

Cholinesterase activity occurs in the epithelium along the length of the pre- and postoral ciliary bands, but not in the telotroch (Fig. 19).



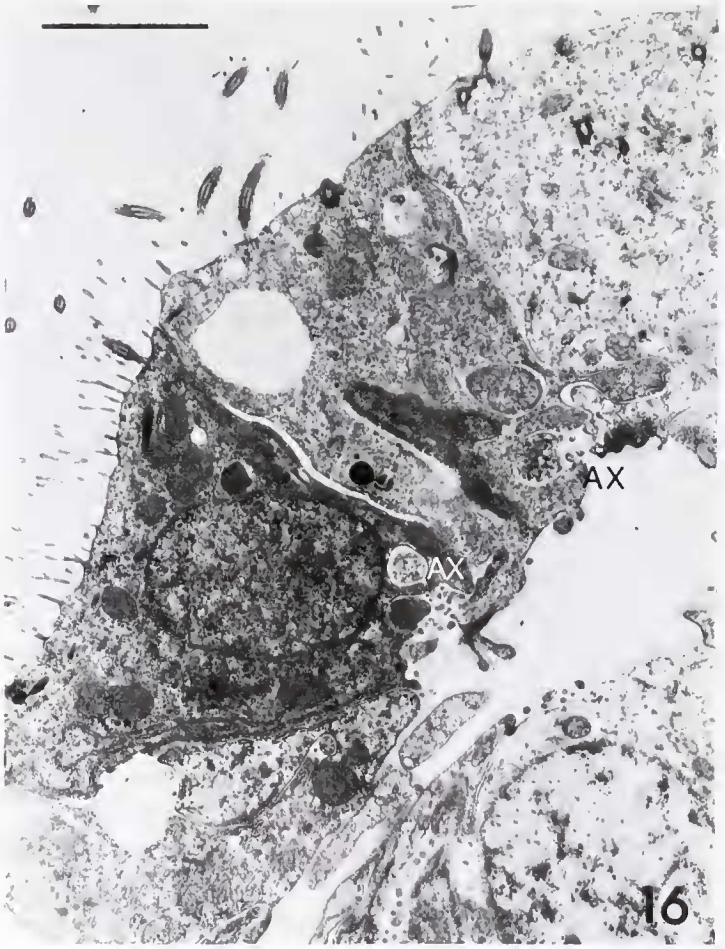
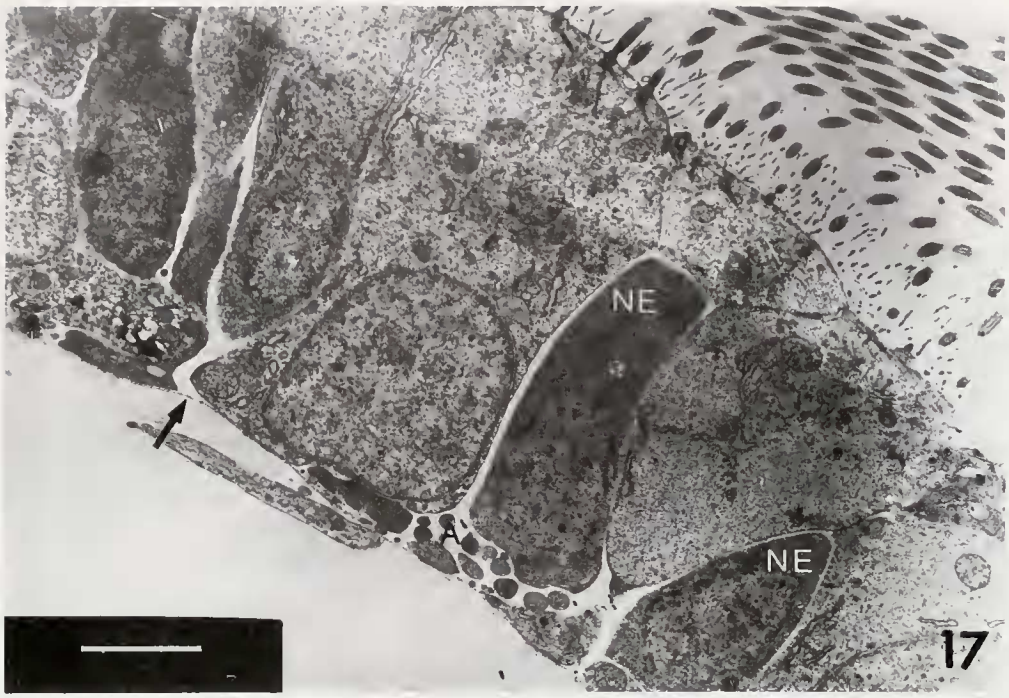


Figure 15. TEM. Epithelium of the stomach. Several axons (A) lie at its base adjacent to basal lamina. Scale bar = 2 μm.

Figure 16. TEM. Longitudinal section of part of the pyloric sphincter with axonal tracts (AX) at the base of the epithelium. Scale bar = 2 μm.



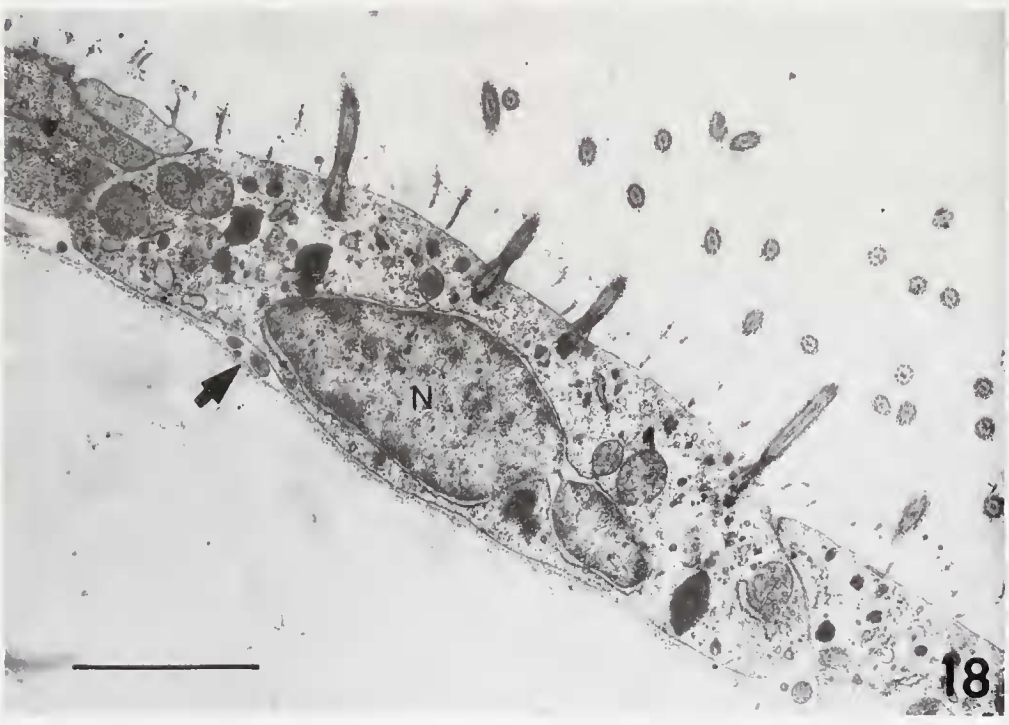
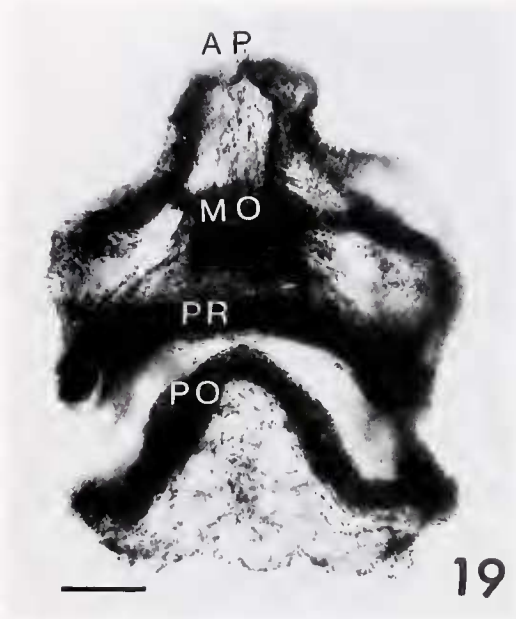
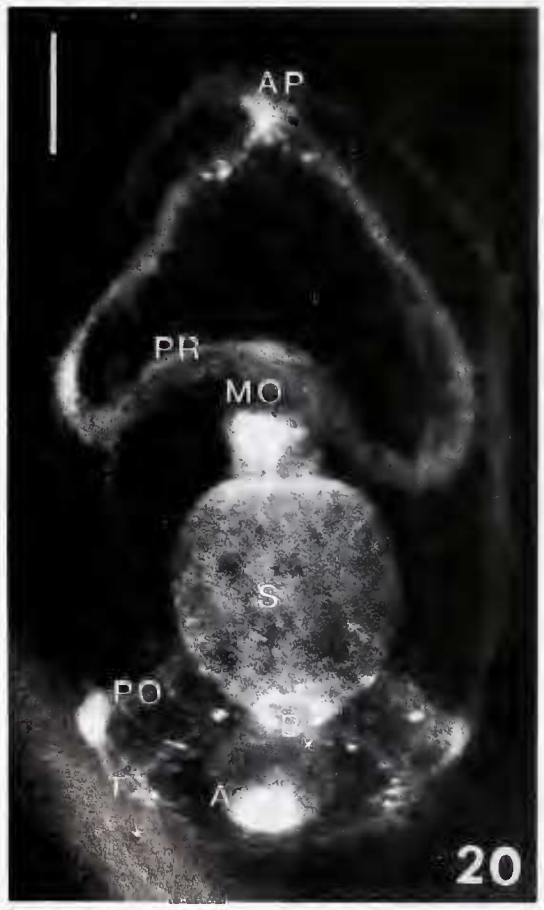


Figure 17. TEM. Cross section of the posterior ciliary band (telotroch) with the axonal tract (A) and two neuron-like cells (NE). Arrow indicates a basal lamina. Scale bar = 2 μm.

Figure 18. TEM. Cross section of the ciliary ring between telotroch and anal opening. Multiciliated cell with lobated nucleus (N) is shown. Axons (arrow) pass between it and its basal lamina. Scale bar = 1 μm.

Bright green fluorescence in glyoxilic acid preparation reveals what appear to be accumulations of the nerves (Fig. 20). The aboral plate appears as a cluster of fluorescent cells at the anterior tip of the larva (Fig. 21). A group of about 30–40 cells lies just between the eyespots. These cells extend their axons subepithelially along ciliary bands. A second group of nerve cells is located in the ventral part of the larva just behind the mouth opening (Fig. 22).





Axons from these cells pass anteriorly and enter the preoral ciliary band forming the circumoral nerve ring. In addition, a tract of axons passes backward in the ventral midline. These axons enter the postoral ciliary band.

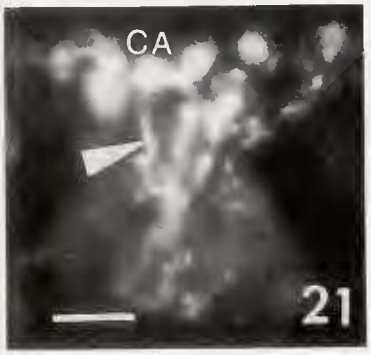
In the stomach wall near the entrance to the intestine, the axons of a group of about ten fluorescent cells form a ring (Fig. 23). Several axons connect this ring with the axonal tract in the postoral band. Fluorescent cells in the epithelium of the telotroch all send axons into the postoral tract too (Fig. 23). In the telotroch itself, no fluorescent axonal tracts were detected. Axonal tracts which pass along the ciliary bands include no more than several fluorescent axons (Fig. 24). Green fluorescing cells in the telotroch send processes to the postoral nerve tract (Fig. 25). Bright nonspecific fluorescence was observed around the anus opening (Figs. 20, 23). No green fluorescing cells or fibers were found there.

Discussion

The tornaria exhibit a coordinated behavior which consists of swimming while permanently rotating about a long body axis and feeding on planktonic unicellular algae or organic particles (Strathmann and Bonar, 1976). Both locomotion and feeding are affected by the activity of the ciliary bands. But unlike echinoderm larvae, the tornaria has a specialized locomotory ciliary band, the telotroch, in addition to the pre- and postoral ciliary bands, which also capture and transfer food particles to the mouth. As in planktotrophic echinoderm larvae, this process is accomplished in tornaria by a local reversal of ciliary beat when food particles are contacted (Strathmann and Bonar, 1971, 1976). Cilia of the telotroch beat synchronously, bringing about a metachronal ciliary wave, but some parts of the telotroch may stop beating while other parts continue. This mechanism is involved in larval orientation and control of locomotion. Coordination of locomotion and feeding allow us to suppose that the behavior of tornaria is controlled by the nervous system. A standard method for identifying certain nerve cells is the histochemical localization of biogenic monoamines—*e.g.*, dopamine, noradrenaline, L-tyrosine, serotonin—by treatment with a solution of glyoxilic acid (Keenan and Koopowitz, 1981). Nerve elements thus found in the tornaria are localized along the ciliary bands. Groups of neuron bodies—*i.e.*, ganglia—lie in the apical plate and behind the mouth opening, and nerve tracts run subepithelially, reflecting the configuration of the ciliary bands. In addition, single catecholamine-containing (CA) cells are

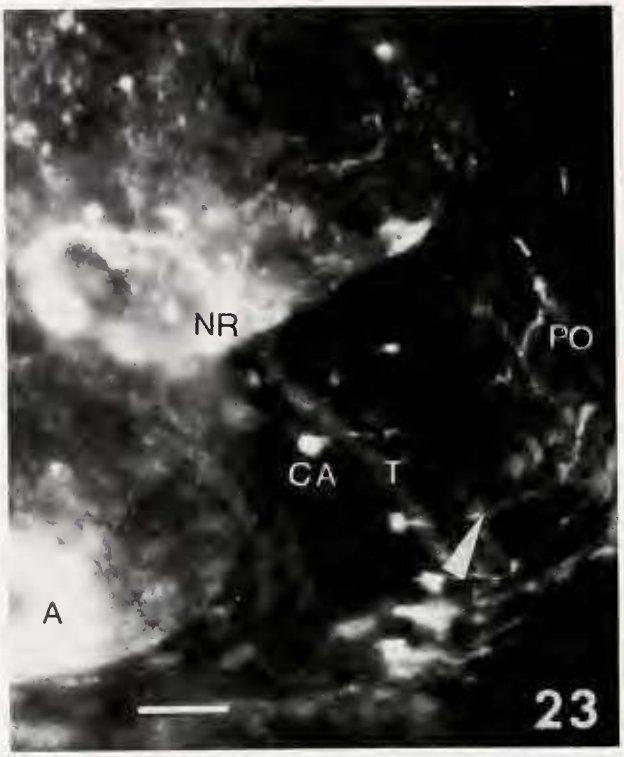
Figure 19. Cholinesterase activity zones (dark parts of the specimen). Ventral view of the entire larva. AP, apical plate; MO, position of the mouth opening; PR, preoral ciliary band; PO, postoral ciliary band. Scale bar = 100 μm.

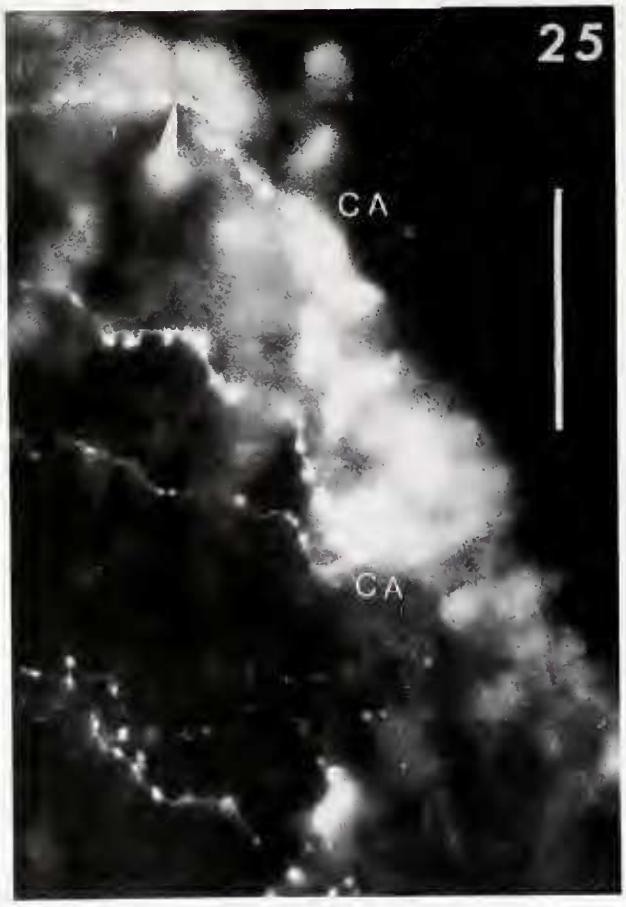
Figure 20. Glyoxilic acid-induced fluorescence (GAF). Total ventral view of entire larva. AP, apical plate; MO, mouth opening; B, border between the stomach and the intestine; S, stomach; T, telotroch; A, anus; PR, preoral ciliary band; PO, postoral ciliary band. Scale bar = 100 μm.


CA
21


CA
22


24


NR
PO
CA
T
A
23


25
CA
CA

localized in epithelia of the postoral ciliary band and the telotroch. Cholinesterase is detected in ciliary bands, excluding the telotroch.

The electron microscopic data agree with the histochemical ones. Subepithelial strands of cell processes were found in the ciliary bands. The ultrastructure of these processes (*i.e.*, the presence of vesicles, microtubules, and mitochondria) clearly suggests that they are axons. Two cell types that send processes to the nervous tracts can be distinguished. The first are ciliated cells found in the epithelia and telotroch, which send axons from their bases. Subepithelial processes originates from the second type of nonciliated cells especially in the region of aboral and esophageal ganglia. The first type of neuron may perform a sensory function, whereas the second type may be an inter- or motoneuron. Similar cells and nerve plexes adjacent to basal lamina were described earlier in the ocelli of the tornaria of *Ptychodera flava* (Branderburger *et al.*, 1973). Unfortunately, no morphologically distinct synapses, either between nerve cells, or between neural and muscle cells, were found in the tornaria. Our assumption that these cells are neural is mainly based on their ultrastructural localization being coincident with the distribution of cells containing biogenic amines (specific neurotransmitters). The apparent absence of synapses is a common feature of marine invertebrate planktotrophic larvae, since no ultrastructural investigations have revealed these structures in pilidia larvae of nemertines (Lacalli and West, 1984), echinoderm larvae (Burke, 1983a, b), or in the actinotrocha larvae of phoronids (Hay-Schmidt, 1989). The detection of catecholamines and of cholinesterase activity suggests that monoamine and cholinergic regulatory mechanisms occur in the tornaria. Evidently there are additional neurotransmitters in the nervous system of tornaria. This is confirmed by the presence of various vesicles in neurons and their axons, and in neuron-like cells and axons, detected in regions of the larva, that exhibit no cholinergic activity and no specific monoamine fluorescence.

In starfish and holothurian larvae, which are morphologically similar to tornaria, the nervous system includes nerve cords lying in the subepithelial layer under ciliary bands and near the esophagus, and many nerve cells containing monoamines lying near the cords (Burke, 1983a; Nezlin *et al.*, 1984; Burke *et al.*, 1986). A similar distribution of neural elements was described in sea urchin larvae (Burke, 1983a; Bisgrove and Burke, 1987). In actinotrochs, intraepithelial nerves pass from the apical ganglion along the midline and around the edge of the epistome (preoral hood). Other nerves are located around the tentacular ring and the posterior ciliary band or telotroch (Hay-Schmidt, 1989). These last neurons, containing catecholamines, are situated in the epithelium along the ciliary bands (Nezlin, 1988). In actinotrochs and tornaria, an apical and a subesophageal mass of nervous cells were detected. In the anterior end of auricularia and bipinnaria, accumulations of catecholamine-containing neurons are absent. Nevertheless, in the "apical ganglion" of auricularia and bipinnaria of several species, massed serotoninergic neurons were shown immunohistochemically (Nakajima, 1988), in one case with antibodies directed against serotonin (Burke *et al.*, 1986).

Despite a strong similarity to echinoderm larvae in ultrastructural localization of neural elements, tornaria differ from them in having catecholamine-containing neurons concentrated in the aboral and esophageal ganglia, and in axons running along the ciliary bands. Posteriorly, solitary catecholamine ciliated cells, connected by axons with nerve tracts, lie in the ciliary band epithelium.

Earlier, Burke (1978, 1983a) suggested that the function of catecholamine-containing neurons in planktotrophic larvae is the coordination of swimming and feeding. Bisgrove and Burke (1987) also pointed out the same possible function for dopaminergic neurons in larval arm epithelia of echinopluteus. The finding of two types of catecholamine cells in tornaria, one concentrated in two subepithelial ganglia sending axons along ciliary bands, and the second scattered in the ciliary band epithelia, allows us to suggest that these cells perform similar functions.

The lack of cholinesterase activity in the telotroch of tornaria probably means that locomotion in tornaria is not acetylcholine dependent. In bipinnaria, cholinesterase is detected throughout the entire ciliary band system. Moreover, in bipinnaria of the asteroid *Lethasterias fusca*, locomotion is controlled by a cholinergic system (Dautov and Semenova, 1988). Thus, the tornaria probably differs

Figure 21. GAF. Catecholamine-containing cells (CA) and their processes (arrow head shows one) in the region of apical plate. Scale bar = 15 μm.

Figure 22. GAF. The cluster of catecholamine containing cells (CA) behind the mouth opening and axonal tract passing from it posteriorly (arrow head). Scale bar = 15 μm.

Figure 23. GAF. Posterior view at the region of postoral ciliary band (PO), telotroch (T), and anus (A). Nerve ring at the junction of stomach and intestine (NR) is shown. Solitary catecholamine containing cells (CA) of telotroch give processes (arrow head shows one) to postoral ciliary band. Scale bar = 15 μm.

Figure 24. GAF. Processes of catecholamine-containing cells (arrow head) in the ciliary band epithelium. Scale bar = 15 μm.

Figure 25. GAF. Catecholamine-containing cells (CA) in the epithelium of telotroch, each having a single process (arrow head). Scale bar = 15 μm.

from the bipinnaria not only in the topology of CA elements but also in the distribution of acetylcholine-containing cells.

In this regard, it is interesting to note that actinotrocha larvae have in their telotroch (a specialized locomotor ciliary band) neither cholinergic- nor catecholamine-containing cells (Nezlin, 1988). Presumably, in the course of adaptive evolution, larvae of certain marine invertebrates—echinoderms, phoronids, and enteropneusts—acquired a set of common morphological and behavioral features. This commonality is especially marked in the bipinnaria and tornaria. Notwithstanding these similarities, however, there are presumed different physiological mechanisms of behavioral regulation.

Literature Cited

Bisgrove, B. W., and R. D. Burke. 1987. Development of the nervous system of the pluteus larva of *Strongylocentrotus droebachiensis*. *Cell Tissue Res.* **248:** 335–343.

Branderburger, I. L., R. M. Woollacott, and R. M. Eakin. 1973. Fine structure of eyespots in tornarian larvae (Phylum: Hemichordata). *Z. Zellforsch.* **142:** 89–102.

Burke, R. D. 1978. The structure of the nervous system of the pluteus larvae of *Strongylocentrotus purpuratus*. *Cell Tissue Res.* **191:** 233–247.

Burke, R. D. 1983a. Development of the larval nervous system of the sand dollar, *Dendraster excentricus*. *Cell Tissue Res.* **229:** 145–154.

Burke, R. D. 1983b. The structure of the larval nervous system of *Pisaster ochraceus* (Echinodermata: Asteroidea). *J. Morphol.* **178:** 23–35.

Burke, R. D., D. G. Brand, and B. W. Bisgrove. 1986. Structure of the nervous system of the auricularia larva of *Parastichopus californicus*. *Biol. Bull.* **170:** 450–460.

Chia, F. S., R. D. Burke, R. Koss, P. V. Mladenov, and S. S. Rumrill. 1986. Fine structure of the doliolaria larva of the feather star *Florometra serratissima* (Echinodermata: Crinoidea), with special emphasis on the nervous system. *J. Morphol.* **189:** 99–120.

Damas, D., and G. Stiastny. 1961. Les larves planctoniques d'Enteropneusts (Tornaria et Planctosphaera). *Mem. Acad. roy. Belgique.* C 1. Sci. 2, ser. **15** (2): 1–70.

Dautov, S. Sh., and M. M. Semenova. 1988. Participation of cholinergic system in the regulation of locomotion of star fishes larvae. Pp. 81–83, in *Prostye Nervnye Sistemy*. Leningrad, Nauka. (in Russian)

Hay-Schmidt. 1989. The nervous system of the actinotrocha larva of *Phoronis muelleri* (Phoronida). *Zoomorphology* **108**(6): 333–352.

Ivanova-Kazas, O. M. 1978. *Comparative Embryology of Invertebrates. Echinoderms and Hemichordates*. Moscow, Nauka. (in Russian)

Keenan, C. L., and H. Koopowitz. 1981. Limitations in identifying neurotransmitters within neurons by fluorescent histochemistry techniques. *Science* **214:** 1151–1152.

Koelle, G. B., and J. S. Friedenwald. 1949. A histochemical method for localizing cholinesterase activity. *Proc. Soc. Exp. Biol. Med.* **70:** 617–622.

Krohn, A. 1854. Beobachtungen uber Echinodermenlarven. *Arch. Anat. Physiol. Wissensch.* 208–213.

Lacalli, T. C., and J. E. West. 1985. The nervous system of a pilidium larva: evidence from electron microscope reconstructions. *Can. J. Zool.* **63:** 1909–1916.

Metschnikoff, E. 1870. Untersuchungen uber die Metamorphose einiger Seethiere. Ueber Tornaria. *Z. Wiss. Zool.*, Bd XXII.

Müller, J. 1850. Über die Larven und die Metamorphose der Echinodermen. *Abhandl. Acad. Wiss.* Berlin, for **1848:** 75–110.

Nakajima, Y. 1987. Localization of catecholaminergic nerves in larval echinoderms. *Zool. Sci. 4* **2:** 293–299.

Nakajima, Y. 1988. Serotoninergic nerve cells of starfish larvae. Pp 235–239 in *Echinoderms Biology*. Burke *et al.*, eds. Balkema, Rotterdam.

Nezlin, L. P. 1988. The development of monoaminergic elements of the nervous system in the actinotroch—planctonic larvae of *Phoronopsis harmeri*. *Journal evolutsyonnoy biochimii i fisiologii 24* **1:** 76–80. (in Russian)

Nezlin, L. P., S. Sh. Dautov, and V. V. Malakhov. 1984. Topography of catecholamine containing neurons in larval development of starfishes. *Doklady Akademii Nauk SSSR.* **278:** 983–985. (in Russian)

Sharp, M. J., and H. J. Atkinson. 1980. Improved visualization of dopaminergic neurons in nematodes using the glyoxilic acid fluorescence method. *J. Zool. Lond.* **190:** 273–284.

Strathmann, R. R., and D. Bonar. 1971. Tornaria's water-working revisited. *Am. Zool.* **11:** 517.

Strathmann, R. R., and D. Bonar. 1976. Ciliary feeding of tornaria larvae of *Ptychodera flava* (Hemichordata: Enteropneusta). *Mar. Biol.* **34:** 317–324.

Strathmann, R. R., T. Jahn, and J. R. C. Fonseca. 1972. Suspension feeding by marine invertebrate larvae: clearance of particles by ciliated bands of a rotifer, pluteus, and trochophore. *Biol. Bull.* **142:** 505–519.

de la Torre, J., and J. Surgeon. 1976. A methodological approach to rapid and sensitive monoamine fluorescence using a modified glyoxilic acid technique: the SPG-method. *Histochem.* **49:** 81–93.

Van der Horst, K. 1933. Enteropneusts of the seas of the USSR. *Issl. morei SSSR* **19:** 73–78.